ORIGINAL ARTICLE

Multiresistant *Pseudomonas aeruginosa* serogroup O:11 outbreak in an intensive care unit

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Objective: To determine whether 15 multiresistant *Pseudomonas aeruginosa* isolates from an intensive care unit (ICU) outbreak were related, were endemic, and belonged to the O:12 European clone.

Methods: Forty-six *P. aeruginosa* isolates from a large hospital were investigated with respect to their antibiotic resistance profiles, serogroups, bacteriocin types and DNA fingerprints obtained by pulsed-field gel electrophoresis (PFGE) of genomic DNA digested with *Xba*I.

Results: Fourteen of the ICU outbreak isolates were indeed identical with respect to their serogroup, O:11, pyocin type, 10/a, and PFGE type, A. Clone A was endemic and dominant throughout the hospital, even though, within the ICU, it underwent phenotypic alterations, such as loss of cell wall lipopolysaccharide side-chains, or acquisition of ceftazidime and imipenem resistance. Bacteriocin typing was more discriminatory than serotyping, but PFGE could differentiate further among phenotypically identical strains. It also allowed the tracking of an O:6 strain, as it was becoming gradually more resistant and undergoing a bacteriocin-type conversion while remaining genotypically unaltered.

Conclusions: Using three typing methods, a nosocomial multiresistant strain distinct from the previously described dominant European O:12 clone was characterized, and the ability of PFGE to identify clonal isolates even when these appear phenotypically distinct was demonstrated.

Key words: *Pseudomonas aeruginosa*, nosocomial infection, antibiotic resistance, serotyping, bacteriocin typing, pulsed-field gel electrophoresis

Pseudomonas aeruginosa is a predominant nosocomial pathogen, ranking among the first three most frequently isolated in intensive care units (ICUs) [1]. It also causes life-threatening infections in other circumstances where patients are immunocompromised [1,2] or have a serious underlying condition, such as cystic fibrosis [3]. The infectious pathogen can be transmitted horizontally within a family [4] or a group of patients [5,6], or derive from a common environmental source [7]. Nosocomial strains are frequently multiresistant, and are notorious for their ability to acquire further resistance mechanisms during antibiotic treatment [8]. Therefore, surveillance, prevention of infection and control of *P. aeruginosa* are of the utmost importance in the hospital environment. The efficiency of such measures depends on the availability of appropriate epidemiologic studies. To this end, several typing systems for discrimination among strains have been used. Phenotypic methods, such as antibiotic resistance profiles, serotyping and bacteriocin typing are useful but are often hampered by poor reproducibility and by the frequency of phenotypic conversion [9]. On the other hand, genotypic methods (DNA fingerprinting), such as hybridization of genomic DNA digested with a frequent-cutting restriction endonuclease, with an rDNA [10] or an exotoxin A gene probe [11], pulsed-field gel electrophoresis (PFGE) of genomic DNA

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digested with a rare-cutting enzyme [12,13], or the random amplification of polymorphic DNA sequences (RAPD) by the polymerase chain reaction [14–16], are highly reproducible and discriminatory [17].

We have studied isolates from an ICU outbreak, together with unrelated isolates from the same hospital, by antibiotic resistance profile, serotyping, bacteriocin typing and PFGE, admitted to be the most discriminatory DNA fingerprinting method for this organism [18]. We report the comparative results of the four methods, which revealed a multiresistant O:11 strain, distinct from the O:12 multiresistant clone previously reported to be dominant in Greece [19] and the rest of Europe [20].

MATERIALS AND METHODS

Bacterial strains

Forty-six *P. aeruginosa* strains were isolated at the Voula General Hospital over the period from 21 February 1995 to 26 March 1996. They were identified by standard microbiological methods (including tests for cytochrome oxidase, arginine dihydrolase, gelatinase, and growth at 42°C) and their identification was confirmed by the API20 NE System (Bio Merieux).

Antibiotic susceptibility testing

Antibiotic susceptibility to the antibiotics listed in Table 1 was determined by the disk diffusion method, according to published standards [21].

Serotyping

Serotyping was performed by agglutination on slides, as previously described [22], using commercially obtained sera (Difco).

Bacteriocin typing

Bacteriocin typing was performed essentially according to Fyfe et al [23], as previously described [24].

Pulsed-field gel electrophoresis of macrorestricted genomic DNA

A modification of three previously published protocols was followed [13,14,25]. Briefly, 600 μL of an overnight bacterial culture in nutrient broth was harvested at 4°C and washed twice in cold phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.5). The cells were resuspended in 300 μL of PBS, to which 20 μL of lysozyme (25 mg/mL) and 300 μL of 2% low-melting-point agarose in PBS was added. After thorough mixing, two 100-μL aliquots for each strain were dispensed in plastic molds (BioRad). After the plugs had set, they were incubated in 500 μL of PBS at 37°C for 1 h. They were rinsed with 1 mL of 0.5 M EDTA, pH 9.0, and then incubated in 500 μL of filter-sterilized ES buffer (0.5 M EDTA, pH 9.0, 1% *N*-lauroylsarcosine) containing 5 μg of proteinase K, overnight at 55°C. They were washed 6×30 min with 850 μL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.6), and one plug for each strain was incubated with 40 u of *Xba*I (New England Biolabs) in a total reaction volume of 250 μL, overnight at 37°C. After digestion, the plugs were equilibrated in ×0.5 TBE buffer (45 mM Tris-borate, 1 mM EDTA) and electrophoresed through a 1% agarose gel in ×0.5 TBE, which had been pre-run for 1 h, using a CHEF DRIII apparatus (BioRad). The gel was run at 14°C, 6 V/cm and 120° switch angle for 18 h, using the linear switch time ramps indicated in each figure legend. It was then stained in 0.5 μg/mL ethidium bromide and photographed (Polaroid) under UV illumination. Lambda phage DNA concatamers (New England Biolabs) were used as DNA size markers. The isolates' chromosomal fingerprints were compared by eye and assigned to PFGE types and subtypes, according to published guidelines [26,27].

Lipopolysaccharide electrophoresis

Cell wall lipopolysaccharides were prepared and separated by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), followed by silver staining, using essentially the Aucken and Pitt [28] modification of the proteinase K method of Hitchcock and Brown [29], as already described [30].

RESULTS

Over the last 9 months of 1995 and during 1996, the frequencies of *P. aeruginosa* isolation in the Voula General Hospital were 14% and 13.7%, respectively, making this organism the second most frequently isolated after *Escherichia coli* (19.7% and 18.9%). One-fifth to 27% of all *P. aeruginosa* strains isolated came from the ICU, where this organism represented 18.9% and 16.9% of total isolates over the same periods, being second only to *Acinetobacter baumannii* (19.8%) in 1995 or *Staphylococcus aureus* (28%) in 1996. During the period from 27 August 1995 to 10 November 1995, 13 *P. aeruginosa* strains that were resistant to all anti-pseudomonal antibiotics were isolated from a total of 49 specimens (47 from the trachea, one from bedsores, and one from urine) originating from 10 patients in the Voula General Hospital, Athens, Greece. Twelve isolates were from the ICU and one was from a patient of the general surgery B ward, who had been moved there from the ICU a month earlier. The ICU patients had a wide variety of clinical conditions, and four of

Table 1 List of strains and summary of results

	Patient No.	Isolate No.	Isolation date	Ward	Specimen of isolation	Resistance phenotype P	Z	I	C	X	A	T	F	Sero-type	Bacteriocin type	PFGE type
	11	Δ 963	21/02/95	ICU	t	P	Z			X	A	T	F	11	10/a	A1
	12	Π 131	24/03/95	OR-E	on									5	11/g	C
	13	Δ 204	15/04/95	ICU	vc	P	Z			X	A	T	F	12	46/1	D
	14	Δ 337	15/05/95	ICU	t	P	Z	I		X	A	T	F	11	10/a	A6
	15	O 569	04/06/95	OR-H	u	P	Z			X	A	T	F	11	10/a	A1
	16	Π 424	15/06/95	OR-E	wx									6	10/s	E
	17	O 748	20/06/95	IM-A	u	P	Z			X	A	T	F	11	10/a	A6
	18	O 879	03/07/95	UR	u	P	Z			X	A	T	F	11	10/a	A5
	19	Π 547	27/07/95	IM-A	bs	P	Z			X	A	T	F	11	10/a	A1
	20	Π 570	03/08/95	OR-Z	bs	P	Z		C	X	A	T	F	11	10/a	A1
	21	O 489	28/08/95	UR	u	P	Z			X	A	T	F	11	10/a	A5
	01	O 194	28/07/95	IM-A	u	P	Z			X	A	T	F	11	10/a	A1
	01	Δ 701	27/08/95	ICU	t	P	Z	I	C	X	A	T	F	11	10/a	A1
	02	Δ 687	21/08/95	ICU	t	P	Z	(I)	C	X	A	T	F	11	10/a	A2
	02	Δ 817	22/09/95	ICU	t	P	Z	I	C	X	A	T	F	11	10/a	A3
	03			GS-B												
		Δ 732	04/09/95	ICU	t	P	Z	I	C	X	A	T	F	11	10/a	A4
	03	Δ 935	17/10/95	S-B	t	P	Z	(I)	C	X	A	T	F	11	10/a	A4
	05	Π 714	15/09/95	ICU	bs	P	Z	I	C	X	A	T	F	11	10/a	A1
	04	Δ 791	18/09/95	ICU	t	P	Z	I	C	X	A	T	F	11	10/a	A4
ICU	08	Δ 823	25/09/95	ICU	t									6	99/p	B
epidemic	08	Δ 857	02/10/95	ICU	t	P	Z	I	C	X	A	T	F	11	10/a	A1
	07	Δ 981	26/10/95	ICU	t	P	Z	I	C	X	A	T	F	11	10/a	A1
	09	Δ 987	26/10/95	ICU	t	P	Z	I	C	X		T	F	PA	10/a	A2
	09	Δ 15	03/11/95	ICU	t	P	Z	I	C	X	A	T	F	11	10/a	A3
	06	Δ 992	30/10/95	ICU	t	P	Z			X	A	T	F	11	10/a	A5
	06	Δ 41	10/11/95	ICU	t	P	Z	I	C	X	A	T	F	11	10/a	A5
	10	Δ 29	07/11/95	ICU	t	P	Z	I	C	X	A	T	F	11	10/a	A5
	10	Δ 40	10/11/95	ICU	t	P	Z	(I)	C	X	A	T	F	11	10/a	A5
	22	O 391	05/11/95	PHY	u	P	Z		C	X	A	T	F	11	10/a	A1
	23	O 823	06/12/95	OR-A	u	P	Z			X	A	T	F	11	10/a	A1
	24	Π 15	11/12/95	OR-B	p					(X)				1	31/k	F
	25	O 886	12/12/95	PHY	u	P	Z	I	C	X	A	T	F	PA	46/h	G
	26	O 935	16/12/95	PHY	u	P			C	(X)	A	T	F	11	10/a	A1
	27	A 921	17/12/95	OR-E	b					(X)				6	11/p	H
	28	Δ 323	10/01/96	ICU	t			I						6	11/y	I
	29	Δ 393	22/01/96	ICU	t	P		I						6	11/y	I
	30	O 429	26/01/96	OR-E	u	P	Z			X	A	T	F	11	10/a	A5
	31	Π 182	04/02/96	OR-E	wx									11	10/a	J
	36	O 673	15/02/96	UR	u	(P)	Z	(I)	C	X	A	T	F	11	10/a	A5
	32	O 800	28/02/96	IM-B	u	P	(Z)		C	X	A	T	(F)	12	1/a	K
	33	no. 63	28/02/96	ICU	a	P		I	C	X			F	6	6/e	I
	37	O 817	29/02/96	IM-B	u					X				2	6/d	L
	34	O 862	04/03/96	OR-E	u	P	Z		C	X	A	T	F	11	10/a	A1
	35	O 943	10/03/96	OR-Z	u	P	Z			X	A	T	F	11	10/a	A1
	38	Δ 687b	19/03/96	ICU	t	P	Z	I					F	6	6/e	I
	39	Δ 82	26/03/96	ICU	a	P	Z	(I)	C	X	A	T	F	11	10/a	A1

ICU=intensive care unit; OR-E=orthopedic E; OR-H=orthopedic H; IM-A=internal medicine A; UR=urology; OR-Z=orthopedic Z; GS-B=general surgery B; S-B=surgery B; PHY=physiotherapy; OR-A=orthopedic A; OR-B=orthopedic B; IM-B=internal medicine B; PA=polyagglutinable.

Specimen codes: t=trachea, on=internal osteosynthesis needle swab, vc=venous catheter swab, u=urine, wx=wound exudate, bs=bedsores, p=pus, b=blood, a=armpit.

Antibiotic codes: (a capital letter designates resistance, and, when within parentheses, intermediate resistance, to the indicated antibiotic): P=piperacillin; Z=aztreonam; I=imipenem; C=ceftazidime; X=cefotaxime; A=amikacin; T=tobramycin; F=ciprofloxacin. All isolates were sensitive to colistin and resistant to chloramphenicol, co-trimoxazole and tetracycline.

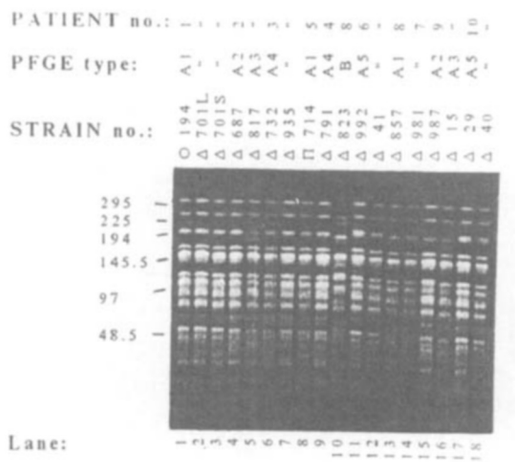

Fig. 1a

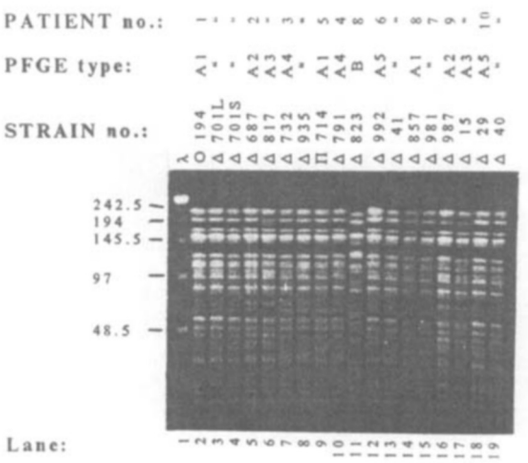

Fig. 1b

Figure 1 Pulsed-field gel electrophoresis patterns of the ICU epidemic isolates. (a) Linear ramp of switch times 0.5 s to 20 s, to resolve the higher-molecular-weight regions of the gel. The sizes, in kb, of λ phage DNA concatamers are indicated to the left of the gel. (b) Linear ramp of switch times 0.5 s to 10 s, to resolve the lower-molecular-weight regions of the gel. The sizes, in kb, of λ phage DNA concatamers are indicated to the left of the gel.

them died during or within 2 months of the epidemic. During the same period, only three more *P. aeruginosa* strains, all from the ICU, were isolated: Δ823 was sensitive to all antibiotics, Δ987 was only sensitive to amikacin, and Δ992 was sensitive only to imipenem and ceftazidime. The possibility that a single strain might be causing an outbreak in the ICU was investigated by serotyping, pyocin typing and PFGE of genomic DNA digested with *XbaI* (Figure 1). As expected, the sensitive isolate Δ823 was distinct by all three methods (Table 1): it was of serogroup O:6, pyocin type 99/p and PFGE type B. All other isolates were grouped together, by serogroup, O:11, pyocin type, 10/a, and PFGE type, A (Table 1). The exception was the amikacin-sensitive isolate Δ987, which differed from the rest only with respect to its O serogroup: it was polyagglutinable, rather than O:11. This difference was explained when cell wall lipopolysaccharide SDS-PAGE was performed (Figure 2). Isolate Δ987 lacked the polysaccharide 'ladder', formed by the O side-chains, displaying instead a pattern characteristic of 'rough' strains (Figure 2, lane 3). In contrast, O:11 isolates, though they differed in their antibiotypes and/or PFGE subtypes, displayed indistinguishable and extensive polysaccharide 'ladders' (Figure 2, lanes 1, 2 and 4). DNA fingerprinting by PFGE allowed further distinctions to be made among isolates belonging to type A, since this could be divided further into subtypes, A1 to A5, differing between them by six, or fewer, bands (Figure 1). For example, A2 differed from A1 in the region between the 97-kb and 48.5-kb markers (Figure 1b, compare lane 5 to lane 4), while A4 had additional differences in the region below the 48.5-kb band (Figure 1b, compare lane 7 to lane 3). A3 yielded an additional fragment below the 194-kb marker (Figure 1a, compare lane 5 to lane 3), while A5 displayed a closely spaced doublet in the 194-kb position (Figure 1a, compare lane 12 to lane 13).

Figure 2 Silver-stained SDS-PAGE of cell wall lipopolysaccharides from representative PFGE type A strains. PA=polyagglutinable.

Since clone A was not restricted to the ICU, but had also appeared in the surgery ward, its dispersion throughout the hospital was examined. In total, therefore, 46 *P. aeruginosa* strains from different wards, from periods both before, during and after the ICU outbreak, and representing 22% of all *P. aeruginosa* strains isolated over the study period, were typed by the same methods. All isolates were nosocomial, using the post-48 h definition, with the exception of O800, which was isolated the next day after patient admission. It was indeed found that clone A was present right from the beginning of the study period, occurring in nine out of 10 wards from which *P. aeruginosa* had been isolated, and in four out of 10 different kinds of specimen (Table 1). However, of the 32 strains that belonged to type A, only 11 that were isolated in the ICU during the August–November 1995 outbreak were resistant to both ceftazidime and imipenem; in other wards, or the ICU at times before or after the outbreak, A strains were sensitive to one or both of these antibiotics (Table 1). Conversely, of the 13 strains that were ceftazidime and imipenem resistant, 11 belonged to type A. The other two were strain O886, of PFGE type G, isolated from a patient on his second day after transfer from a different hospital, and no. 63, the third strain isolated belonging to type I: this clone had been followed as it was gradually acquiring resistance traits (Table 1).

Overall, 17 antibiotypes (considering intermediate resistance as a distinct phenotype), seven O serogroups, 12 pyocin types, and 12 PFGE types plus six subtypes (Figure 3) were observed among the 46 strains.

Serogroup O:11 was prevalent, including 32 of the 46 isolates (70%), with 31 of these belonging to PFGE type A, and one to type J. Serogroup O:6 was represented by seven strains (15%), only two of which were multiresistant. Only two strains (4%), both multiresistant, belonged to serogroup O:12.

Resistance rates to the antibiotics tested ranged from 74% to 83%, with the exception of imipenem and ceftazidime, for which they were 37% and 50%, respectively. However, unlike resistance rates of all other antibiotics, which did not vary greatly among different hospital wards, imipenem and ceftazidime resistance was much more noted in the ICU (70% and 65%, respectively) than elsewhere (4% and 3.5%). In other words, 94% of all imipenem-resistant and 65% of ceftazidime-resistant isolates occurred within the ICU. The resistance phenotypes of all isolates studied are shown in Table 1. In addition to the antibiotics indicated, resistance to timentin (TIM) followed that to aztreonam (AZT), with the exception of isolates O800 (TIM^R/AZT^I), no. 63 (TIM^R/AZT^S), and Δ687b (TIM^S/AZT^R). Gentamicin (GEN) resistance followed

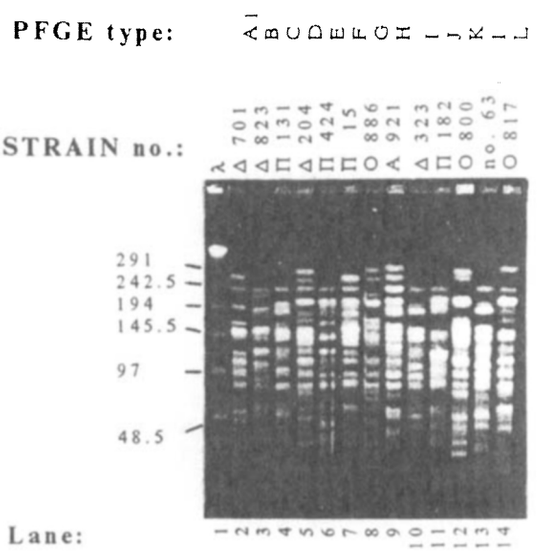

Figure 3 Strains representing all PFGE types observed throughout the hospital during the study period. Linear ramp of switch times 0.5 s to 20 s. The sizes, in kb, of λ phage DNA concatamers are indicated to the left of the gel.

that to amikacin (AMK), with the exception of isolate Δ987 (GEN^I/AMK^S), netilmicin followed tobramycin, while pefloxacin, ofloxacin and norfloxacin followed ciprofloxacin, with the exception of isolates Δ687b, which was sensitive to the latter two only, and isolate O817, which was sensitive to ciprofloxacin only.

With respect to DNA fingerprinting by PFGE, 32 of 46 (70%) isolates belonged to type A; within this type, subtypes A1 (47%) and A5 (25%) were dominant. All A isolates were multiresistant, serogroup O:11, bacteriocin type 10/a, with the exception of Δ987, which was polyagglutinable and pyocin type 10/a. Types B to L were each represented by only one strain, with the exception of I, grouping four isolates (9%). Types A, D, G, I, K and L (six types, 38 strains) corresponded to multiresistant isolates, contrary to types B, C, E, F, H and J, which grouped more sensitive strains (six types, six strains).

PFGE type A was dominant (74% of isolates) in the ICU, followed by I (23%), while B and D were each represented by one strain. The greatest genotypic variety was obtained in the orthopedic E ward (PFGE types A (two strains), C (one strain), E (one strain), H (one strain), J (one strain)).

The majority of strains were isolated from the trachea (50%), followed by urine (33%) and various wound exudates (13%). In all cases, PFGE type A was prevalent, representing 78%, 80% and 50% of isolates from the above specimens, respectively.

DISCUSSION

A nosocomial multiresistant *P. aeruginosa* O:11 strain was identified, which not only caused the ICU epidemic but was also widespread in the rest of the hospital. The fact that a second outbreak was not observed was attributed to the reinforcement of strict hygiene measures taken to exclude transmission via hospital personnel, and the thorough decontamination of the ICU ward and all equipment used.

Typing by PFGE afforded the highest discrimination among strains. Although it would appear that antibiotic susceptibility was equally discriminatory, having yielded the same number of types, 17, it was clearly misleading as to strain identity, since, for example, it could not discriminate between Δ963 and Δ204, which differed by all other methods. Pyocin typing (12 types) was almost twice as efficient as serotyping (seven types) in distinguishing between strains and equal to DNA fingerprinting by PFGE (12 types). However, the latter method could divide the dominant type further into six subtypes, thus significantly increasing discriminatory ability. The subtle variations in the genomic macrorestriction patterns of different A subtypes did not correlate with phenotypic differences and were such as can be expected from closely related *P. aeruginosa* strains, including consecutive isolates from the same patient [14, 31].

Furthermore, and most importantly for the epidemiologic surveillance of antibiotic resistance, only PFGE allowed us to follow a specific clone, I, as it was gradually evolving towards a more resistant phenotype (Table 1). This clone was represented by four genotypically indistinguishable isolates, Δ323, Δ393, no. 63 and Δ687b, all from the ICU and from different patients. The first two isolates were phenotypically identical, except with respect to their susceptibility to piperacillin. However, when the next strain, no. 63, was isolated 5 weeks later, the pyocin type had changed from 11/y to 6/e, as was the case for the last isolate of the same clone, Δ687b. This change was concomitant with a change in antibiotype, as the latter strains were resistant to other β-lactams and to quinolones, contrary to the former two. It would appear that no. 63 and Δ687b had both diverged independently from Δ393, rather than being sequential stages of a single evolving strain, since no. 63 was resistant to all quinolones and cephalosporins, contrary to the later isolate, Δ687b.

PFGE revealed a greater genetic homogeneity among multiresistant isolates, compared with the rest. This is to be expected in a hospital environment, where the pressure of antibiotic usage favors the selection and clonal expansion of resistant strains. Indeed, multiresistant clone A was endemic in the hospital: it had been present before the ICU outbreak and persisted after it. An A1 strain even managed to replace, within a week, the sensitive strain B carried by patient 08.

It was interesting to note that neither clone I nor the more widespread clone A belonged to the 'typical' serogroup for multiresistant strains, O:12, which has been shown to be widespread in Greece [19], as in Europe [20]. In fact, there were only two O:12 strains isolated in our hospital during the study period, and these were indeed multiresistant. A Greek multicenter study, however, has shown that the clinically prevalent serogroups are precisely O:11 and O:12, both largely associated with multiresistant phenotypes (unpublished results). In the past, serogroup O:11 had been described as one of the most common, both from environmental water sources [32–34] and in hospital epidemics [35–37], across Europe and in the USA, but, in general, had not been associated with high resistance rates. Specifically, 15 years ago in Greece, O:11 had been described as a generally antibiotic-sensitive serogroup, unlike O:12 [38]. However, there have been recent reports of O:11 strains exhibiting resistance to aminoglycosides [39] or aminoglycosides and quinolones [40], but not to β-lactams, as in the present study. Indeed, in our hospital, resistance to β-lactams was always crossed to both aminoglycosides and quinolones, with the exception of the PFGE type I isolates, and the polyagglutinable strain Δ987. Finally, whereas endemic clone A was generally sensitive to ceftazidime and imipenem, within the ICU it developed resistance to these antibiotics, presumably because of their long-term use in that environment.

Since all except one of the ICU outbreak isolates came from the trachea, a common site of colonization, it is impossible to tell whether clone A was causing an infection in ICU patients already suffering from serious underlying conditions. However, it has been proposed recently [41] that carrier status should be seriously taken into account in a definition and surveillance of nosocomial infections; at any rate, A strains were clearly infectious in wards outside the ICU. Moreover, the evolution of a strain, belonging to a serogroup previously considered susceptible, to a totally resistant state, including resistance to imipenem, is cause for alarm. Development of resistance during antibiotic treatment, especially within an ICU and for *P. aeruginosa* [42], is, of course, a well-known phenomenon, which has been described for various antibiotics [43], including β-lactams [44–46]. Such an evolution was traced most strikingly with strains Δ323, Δ393, no. 63 and Δ687b. These genotypically indistinguishable (PFGE type I) isolates were all phenotypically O:6, one of the most common clinically encountered serogroups [35,38,47]. However, in the space of 2 months, clone I had developed from resistance to only one antibiotic

to multi-resistance. Parallel with its acquisition of resistance traits, its pyocin type changed from 11/y to 6/e. Phenotypic changes unaccompanied by a change in genotype are a frequent observation in strains which persist in the human host, such as those infecting cystic fibrosis patients [48,49]; long-term tracheal colonization in an ICU setting can be thought of as a similar mode of persistence. Another similarity between the two conditions is illustrated by isolate Δ987 of our study. This converted to a polyagglutinable serogroup, by losing its polysaccharide side-chains, while remaining genotypically indistinguishable from other clone A strains. Conversion to polyagglutinability is widespread among cystic fibrosis *P. aeruginosa* strains, and is attributed to their long-term persistence in the lungs [4], though loss of lipopolysaccharide side-chains has also been noted in other chronic colonization contexts [50].

In conclusion, we have characterized a multiresistant O:11 strain, distinct from the dominant European multiresistant O:12 clone, by serotyping, bacteriocin typing and PFGE. The last method also allowed us to trace an O:6 clone as it was gradually becoming more resistant, and underwent a bacteriocin-type conversion, within the ICU. This study therefore presented two striking examples of the power and usefulness of PFGE in a nosocomial environment, not only in the investigation of outbreaks, but also in the monitoring of strain evolution and dissemination.

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